The Role of Taxonomic and Functional Macroinvertebrate Diversity as Indicators of Nutrient Pollution in Ohio Streams

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Abstract

Nutrient pollution of United States surface waters, primarily due to agricultural and urban runoff, is currently one of the biggest contributors to the impairment of aquatic ecosystems. Sensitivity of aquatic macroinvertebrates to environmental stressors (e.g. excess nutrients) has made them historic indicators of water quality. Taxonomic indices are commonly used to represent macroinvertebrate diversity, however, the use of functional traits as a diversity measure has become increasingly popular due to their ability to mechanistically link macroinvertebrate communities to environmental stressors. The objectives of this research project were to 1) quantify macroinvertebrate diversity across watersheds of varying land use (e.g. agricultural, forested, and mixed use) and 2) across watersheds, examine whether more variation in nutrients is explained by either taxonomic or functional macroinvertebrate diversity measures. I predicted that areas experiencing high nutrient pollution would have low diversity values (e.g. taxonomic and functional) while areas characterized by low to moderate nutrient pollution would have high functional and taxonomic diversity. I found that taxonomic diversity and richness did not significantly differ across watersheds, however, linear regressions revealed a positive and significant relationship between richness metrics and phosphorus concentrations. Functional feeding group richness differed significantly across watersheds and further analysis of functional groups provided insight as to potential environmental stressors impacting macroinvertebrate communities. Integration of functional metrics alongside taxonomic indices can help identify locations that have been negatively impacted by nutrient pollution and could help pinpoint areas in which management strategies would be most effective at improving the overall function of a watershed.

Introduction

Background and Justification

Anthropogenic disturbances as a result of increased pressures to alter forested land for agricultural and urban use can have significant detrimental impacts on aquatic ecosystems (Karr, 1999). In the past 70 years, over 40 percent of land in the world has been altered from its original state (Vandewalle et al., 2010). It is estimated that up to 40 percent of surface water contamination is caused by nitrogen and phosphorus runoff from non-point sources of pollution (e.g. agriculture and residential urbanization) (Justus, Petersen, Femmer, Davis, & Wallace, 2010; Wang, Robertson, & Garrison, 2007). As a direct result, many scientists cite nutrient pollution as a prominent threat to environmental degradation, as increased nutrient inputs can negatively impact both biotic and abiotic components of an ecosystem (Camargo, Alonso, & De La Puente, 2004; Smith, Bode, & Kleppel, 2007).

Converting forests to cropland or pasture for livestock reduces the amount of in-stream canopy cover, replaces native perennial species (e.g. trees, vegetation) with annuals that do not have the potential to store nutrients year-round, and reduces habitat heterogeneity within streams. Similarly, urbanization converts permeable surfaces to impermeable surfaces, reducing groundwater recharge and allowing precipitation runoff to be transported across a watershed more quickly than would occur in an undisturbed system (Johnson, Jin, Carreiro, & Jack, 2013). In both cases (e.g. conversion to agriculture or impermeable surfaces), nutrients can act as contaminants to aquatic ecosystems when excess amounts are transported to surface waters via runoff. High nutrient concentrations accumulate as water flows downstream, eventually meeting a lentic system (e.g. lakes and reservoirs) and potentially contributing to eutrophication. If conditions are optimal for algal growth, nutrient loading to lentic waters can cause harmful algal

blooms that are a significant risk to public health due to production of hazardous cyanobacterial toxins (Delong & Brusven, 1998; Wallace & Webster, 1996; Wang et al., 2007).

Excess nutrients in aquatic systems have been shown to have detrimental effects on the biotic integrity of a watershed and can cause permanent shifts in stream productivity and nutrient cycling (Johnson et al., 2013; Stewart, Butcher, & Swinford, 2000). Forested streams typically derive a large portion of their energy from allochthonous inputs such as large woody debris and detritus. When land is converted to agricultural or urban use, riparian area is often lost or greatly reduced and nutrient inputs to the system increase (Stewart et al., 2000). High nutrient concentrations can act as an environmental stressor to aquatic organisms, leading to changes in taxonomic diversity and shifts in the functional composition of a community (Chessman & McEvoy, 2012). One such consequence is a shift towards communities containing organisms that obtain more of their energy from autochthonous sources found within the stream, such as algae and phytoplankton (Barton & Metcalfe-Smith, 1992). Given the impacts that nutrient pollution can have on aquatic ecosystems and the services they provide, scientists and regulators are frequently trying to identify the best indicators of surface water impairment.

Chemical water sampling is frequently employed to monitor stream impairment, however, this method provides only a snapshot of current conditions. In contrast, biological indicators (e.g. fish and macroinvertebrates) provide a more reliable description of aquatic impairment because they reflect a longer-term signature of environmental stressors affecting the ecosystem (Song et al., 2009). In order to protect public and aquatic health, reduce the risk of harmful algal blooms, and protect economic services derived from aquatic ecosystems, it is important to develop a sound method which will allow management professionals to pin-point

areas in streams where non-point source pollution is most prevalent. This will ultimately aide in identification of areas of improvement in watersheds and indicate areas in which non-point source management practices would be most effective at reducing nutrient loads.

Aquatic macroinvertebrates have historically been used as a biotic indicator of water quality impairment due to their sensitivity and responsiveness to numerous environmental stressors. Differences in organisms' sensitivity to nutrient pollution allow scientists to more accurately observe changes in the chemical environment over a period of time (Camargo et al., 2004; Charvet, Kosmala, & Statzner, 1998). For this reason, macroinvertebrates are extremely useful in monitoring the impact of nitrogen and phosphorus pollution. Multiple studies have shown a strong connection between in-stream nutrient concentrations and macroinvertebrate diversity and abundance (Beketov, 2004; Blinn & Ruiter, 2013; Song et al., 2009). Streams exhibiting low to moderate amounts of available nutrients tend to have high biodiversity and a wide-ranging community of tolerant and intolerant taxa. Streams with a high amount of nutrients tend to have lower biodiversity of organisms, few intolerant taxa, and are dominated by tolerant taxa (Smith et al., 2007; Song et al., 2009; Wang et al., 2007).

Although macroinvertebrate indices have been very useful in identifying and monitoring impairments in aquatic ecosystems, most indices focus largely on describing taxonomic richness and diversity. Recent research focused on quantifying functional trait diversity suggests that incorporating life-history traits into analyses can provide a mechanistic link or explanation that is often missing from taxonomic diversity indices (Dolédec, Phillips, Scarsbrook, Riley, & Townsend, 2006; Pollard & Yuan, 2009; Wallace & Webster, 1996). For example, per the river continuum concept, macroinvertebrate assemblages have been observed to follow a distinct

composition along a longitudinal gradient. Organisms are distributed according to functional feeding groups such that heterotrophic communities (e.g. consumers of allochthonous material) are found in headwaters of streams, transitioning to more autotrophic communities (e.g. consumers of algae) along a longitudinal downstream progression (Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Environmental stressors, such as nutrient pollution, can cause a shift in the composition of macroinvertebrate communities present within a stream reach. Incorporating life-history traits, such as functional feeding groups and modes of existence (i.e. an organism's method of movement, attachment, or concealment), can help scientists understand the connections between community composition, habitat stressors, and nutrient concentrations (Merritt & Cummins, 2008). This method can help to reveal the effect of land use changes and nutrient loading on macroinvertebrate communities while concomitantly explaining variation (Delong & Brusven, 1998; Vandewalle et al., 2010; Verberk, van Noordwijk, & Hildrew, 2013).

Taxonomic Diversity

Taxonomic diversity measures are used universally as an indication of biotic health because they are easy to calculate and cost effective. Federal and state agencies frequently use taxonomic methods because they produce a single, comparable value that is applicable across many regions. These indices include Shannon Diversity (H'), Simpson's Reciprocal Index (1/D), and the Invertebrate Community Index (ICI).

Shannon Diversity (H') produces a value describing species abundance and evenness. Research has shown that H' typically decreases with increasing environmental stress (Charvet et al., 1998). Beketov (2004) observed that Shannon Diversity values for mayflies decreased when ammonia and nitrate nitrogen concentrations increased. In an evaluation of agricultural sites

varying in nitrate concentrations, Song et al. (2009) found that Shannon Diversity values decreased when nitrate concentrations increased. Similar to Shannon Diversity, Simpson's Reciprocal Index (1/D) is a measure of species richness and relative abundance and is predicted to decrease with increasing levels of disturbance (e.g. elevated nutrient concentrations). Researchers have hypothesized that Simpson's and Shannon's indices result in overestimations of diversity because of their inability to account for individual organism's exhibited tolerances. For example, a site dominated by nutrient-tolerant taxa (e.g. oligochaetes, gastropoda) may produce a score identical to a site dominated by intolerant taxa (Weigel & Dimick, 2011). Charvet et al. (1998) claim that the Shannon Diversity value obtained is also heavily dependent on sampling method or the type of equipment used and varies greatly between seasons (Charvet et al., 1998). These limitations could prove counterproductive when attempting to evaluate the effects of environmental stressors on macroinvertebrate communities for the reasons listed above.

The Invertebrate Community Index, developed by DeShon (1995), is commonly used by federal and state agencies to quantify macroinvertebrate diversity. The index contains a total of ten metrics, the majority of which evaluate taxonomic diversity by calculating relative abundances or absolute numbers of organisms. Complete calculation of the ICI requires deployment of Hester-Dendy artificial substrate samplers and a 6-week waiting period to allow for colonization of individual plates. After the 6-week period, organisms are counted and identified to the lowest possible taxonomic level to obtain a quantitative measure of macroinvertebrate diversity (DeShon, 1995). The process of obtaining data for the ICI is time consuming and takes longer to acquire than other methods of sampling (e.g. Surber, Ekman), however, it provides a means to quantify relative abundances of organisms to produces a single

value that is easily comparable across regions. In a study of over 900 streams, Miltner and Rankin (1998) found that ICI scores were negatively correlated with total nitrogen and total phosphorus concentrations. The data contained a great deal of variation only of which 20-30 percent could be attributed to differences in habitat. The variation in ICI scores led the scientists to conclude that calculating trait-based metrics in addition to taxonomic metrics would help provide a better explanation of the observed variation (Miltner & Rankin, 1998).

Functional Diversity

Functional diversity metrics have become increasingly popular as a method of evaluating the effect of environmental disturbances (e.g. changes in land use, nutrient pollution) on macroinvertebrate populations (Usseglio-Polatera, Bournaud, Richoux, & Tachet, 2000; Vandewalle et al., 2010; Verberk et al., 2013). Functional assessments often include the use of functional feeding groups (FFGs) and modes of existence as measures of diversity (Wallace & Webster, 1996). These life-history traits can provide a better explanation of variation between impacted macroinvertebrate communities because they incorporate morphological and behavioral adaptations of organisms into analyses, offering insight into the presence or absence of a particular species group (Camargo et al., 2004).

The River Continuum Concept, introduced by Vannote et. al. (1980) and illustrated in Figure 1, provides a detailed description of the longitudinal progression of macroinvertebrate communities operating at equilibrium in stream ecosystems. Heterotrophic communities dominate headwaters, receiving the majority of their energy from allochthonous inputs (e.g. woody debris and leaves). These stream reaches are characterized by densely forested riparian zones and receive greater amounts of allochthonous material than they are able to consume. The

most prominent FFG in headwaters is shredders because they have the ability to breakdown allochthonous sources of energy to coarse particulate organic matter (CPOM). Shredders make CPOM available to the second most prominent FFG in headwaters, collectors, in the form of fine particulate organic matter (FPOM). Further downstream, heterotrophic communities transition to autotrophic, receiving the majority of their energy from autochthonous sources (e.g. algae and phytoplankton). These streams are characterized by greater channel width, less allocthonous inputs, less canopy cover, and increased sunlight penetration that helps support autochthonous production. These streams are dominated by collectors due to the increased abundance of FPOM for consumption (Vannote et al., 1980).

Land use changes can lead to shifts in macroinvertebrate communities comparatively to what is expected and can significantly alter energy exchanges in aquatic ecosystems. Incorporating FFGs as a diversity metric can identify areas experiencing nutrient impairment based on the composition of consumers found at a particular location. For example, reduction of riparian width in headwaters decreases allochthonous inputs and causes a decline in the shredder organisms that are typically abundant in low order streams (Delong & Brusven, 1998). Loss of allochthonous energy inputs can lead to homogenization of macroinvertebrate communities such that they contain a greater percentage of collectors and scrapers (Barton & Metcalfe-Smith, 1992). These compositional changes are similar to those predicted to occur much further downstream where autochthonous inputs dominate (Delong & Brusven, 1998). Scrapers feed on algae and are found in greatest abundance in reaches between headwaters and larger rivers because widening of the channel supports a sparse canopy that allows for penetration of light for algal growth. Addition of nitrogen and phosphorus to aquatic systems can initiate algal production in-stream and may contribute to higher abundances of scraper organisms in streams

impacted by changes in land use (Johnson et al., 2013). In addition to causing a shift in FFG composition, clearing of riparian vegetation allows greater quantities of nutrients to be deposited into streams. Riparian vegetation is important to nitrogen and phosphorus uptake (Delong & Brusven, 1998; Justus et al., 2010). Macroinvertebrates classified as collectors and scrapers tolerate pollution more readily than organisms found in other FFGs and thus tend to dominate when nutrients concentrations are high (Ashton, Morgan, & Stranko, 2014).

Developing land for urban use or converting forested land for agriculture can lead to increased fine sediment deposition in aquatic systems. In excess amounts, fine sediment can suffocate macroinvertebrates, alter habitat, and fill interstitial spaces that organisms rely on for refuge or spawning habitat (Barton & Metcalfe-Smith, 1992; Piggott, Townsend, & Matthaei, 2015). Analyzing modes of existence (e.g. burrowers, clingers, and swimmers) can help predict levels of impairment from agricultural and urban inputs. Pollard and Yuan (2010) found that addition of fine sediment to tanks containing macroinvertebrates led to a reduction in the amount of clinger species. Wagenhoff et al. (2012) completed a mesocosm experiment in which sediment was added to tanks containing macroinvertebrates for a period of 21 days. At the end of the experiment, researchers concluded that sediment acted as a subsidy to macroinvertebrates in low to moderate amounts but caused a reduction in diversity at high amounts (Wagenhoff, Townsend, & Matthaei, 2012).

Functional diversity is difficult to quantify and obtaining accurate data on FFGs and mode of existence for individual species is time consuming. The use of life-history traits in diversity surveys do not necessarily directly link macroinvertebrate community diversity to nutrient impairment, rather, they provide a more in-depth interpretation of the relevant

environmental stressors affecting the biotic community due to changes in land use. While taxonomic diversity is easier to calculate and more widely used, many researchers have hypothesized that taxonomic metrics tend to provide an over or under estimation of diversity. The variation associated with using taxonomic indices as a sole indicator of diversity has facilitated support for the development of an index that considers both taxonomic and functional diversity (Lücke & Johnson, 2009; Pollard & Yuan, 2009; Usseglio-Polatera et al., 2000). Considering functional diversity when examining taxonomic diversity indices will ultimately lead to better understanding of the effects of nutrient impairment on aquatic macroinvertebrate diversity. Diversity indices offering this level of detail could help managers implement restoration, mitigation, and best management practices targeted towards an individual environmental stressor (e.g. nutrients, sedimentation, etc.). In turn, this would reduce the economic impacts often associated with management and restoration activities.

Hypothesis and Predictions

The goals of this study were to: 1) to examine whether macroinvertebrate diversity indices differed across three watersheds that vary in land use and 2) to determine whether taxonomic or functional macroinvertebrate diversity indices are better indicators of nutrient pollution. Specifically, my objectives were to: 1) quantify and compare nutrient concentrations and macroinvertebrate diversity between an agricultural, forested, and mixed use watershed, and 2) across watersheds, examine whether more variation in nutrients is explained by either taxonomic or functional macroinvertebrate diversity measures. I predicted that areas experiencing high levels of nutrient pollution would have low functional and taxonomic diversity

values and areas characterized by low to moderate nutrient pollution would have high functional and taxonomic diversity (Smith et al., 2007; Song et al., 2009; Wang et al., 2007).

Methods

Study Sites

In order to evaluate the effect of land use on macroinvertebrate diversity, data were collected in streams of three Ohio watersheds that had distinct separation of primary land use (Figure 2). The three study watersheds were chosen based on known differences in land use (e.g. agricultural, mixed use, forested) and nutrient concentrations. Individual study sites within each watershed served as replicates.

Sunday Creek watershed (357 km^2) is located in Southeastern Ohio and is mostly enclosed within a portion of Wayne National Forest. Surface water from streams located within this catchment reaches its eventual deposition into Burr Oak Reservoir (2.6 km^2). It is for this reason that the group of data collected within the Sunday Creek watershed will hereby be referred to as Burr Oak (n=6). The Upper Great Miami River watershed (1937 km^2) in Central Northwestern Ohio is surrounded primarily by agricultural croplands. Encompassing five counties, just over nine percent of the land in the entire catchment has been developed (OEPA, 2012). The majority of streams are bordered with cropland, have little riparian buffer, and contain tile drains from agricultural operations. Surface water from streams located within the Upper Great Miami River watershed is deposited into Indian Lake (20.7 km^2). The group of data collected from the Upper Great Miami River watershed will hereby be referred to as Indian Lake (n=11). Big Walnut Creek watershed (1443 km^2), located in Central Ohio, is classified as mixed use. Most of the catchment's tributaries flow through the city of Sunbury, Ohio or rural and

suburban residential property exhibiting variable physical characteristics. Riparian width fluctuates considerably throughout the watershed. Surface water from streams located within Big Walnut Creek watershed is deposited into Hoover Reservoir (13 km^2). Data collected within this catchment will hereby be referred to as Hoover (n=11). Data for this study was collected in conjunction with a larger research project that is broadly investigating the sources and fates of nutrients in Ohio watersheds.

Chemical Water Quality

Although watersheds were chosen based on known differences in nutrient concentrations I compared nutrient concentrations to validate differences across the three watersheds. Water samples were collected according to methods outlined in the Handbook for Sampling and Sample Preservation of Water and Wastewater (US EPA & Environmental Monitoring and Support Laboratory, Cincinnati, 1982). From July-September of 2016, grab samples were taken at each site by submerging an acid-washed sample bottle in the center of the stream channel. Bottles were immediately placed on ice in the field, frozen upon return to the laboratory, and transported to The Ohio State University's Star Lab in Wooster, Ohio for analysis. Samples were analyzed by Star Lab for total nitrogen (TN), total phosphorus (TP), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), and phosphate-phosphorus (PO_4-P).

Physical Characteristics

Primary land use at each individual site was determined based on direct observations in combination with stream habitat evaluations. To determine differences in the quality of available macroinvertebrate habitat at each site, stream habitat was assessed using the Qualitative Habitat Evaluation Index (QHEI) as defined in Methods for Assessing Habitat in Flowing Waters: Using

the Qualitative Habitat Evaluation Index (QHEI) (Midwest Biodiversity Institute, 2006). The QHEI was developed by the Ohio EPA and the Midwest Biodiversity Institute to describe habitat characteristics of a stream reach (e.g. substrate quality, in-stream cover, riffle/run quality, etc.). Successful calculation of overall QHEI score is dependent on gathering detailed qualitative information during the assessment. Depth at one Hoover site and four Indian Lake sites was too deep to gather accurate data on the substrate type (i.e. streams were unwadeable and were sampled by boat). Visual observations allowed for scoring of the majority of the remaining QHEI metrics at these sites, however, an overall score could not be calculated because substrate data was not recorded.

To evaluate whether riparian width differed between sites in each watershed, I developed an index for quantifiable comparison similar to the one outlined in Song et al. (2009). Scoring was based off of observations recorded for the Riparian Zone metric of the QHEI. In this metric, riparian width is recorded for the left and the right side of the stream bank in five increments (i.e. none, <5 meters, 5-10 meters, 10-50 meters, and >50 meters) (Midwest Biodiversity Institute, 2006). By assigning the left and right bank a number based off of the recorded observation, I was able to average the two scores to produce a value that represented riparian zone width at each site. This value increases with riparian width, with four being the highest score possible (e.g. both stream banks have >50 meters of riparian area) and zero being the lowest score possible (e.g. both stream banks have no riparian area) (See table 1 for scoring system).

Macroinvertebrates

To quantify benthic macroinvertebrate diversity, macroinvertebrate samples were collected according to methods outlined in the US EPA's Rapid Bioassessment Protocols for Use

in Streams and Wadeable Rivers (Barbour, Gerritsen, Snyder, & Stribling, 1999). Sampling occurred once at each study site from July-September of 2016. Macroinvertebrates were collected from riffles in wadeable streams using a Surber sampler (500 μM mesh net, 0.009 m^2) for a 90 second period of effort. One stream at Hoover and four streams at Indian Lake required the use of an Ekman sampler (0.00354 m^3) because the water depth was greater than recommended for Surber sampler use. Due to differences in equipment area, density relationships between sites were not examined. All contents (e.g. detritus, macroinvertebrates, substrate, etc.) of the surber or Ekman sample were placed directly into a bottle and preserved in the field with 70% Ethanol solution. Samples were processed in the laboratory at The Ohio State University, where macroinvertebrates were separated from organic matter and placed into glass vials containing a 70% Ethanol solution. Vials containing the pre-sorted macroinvertebrates were packaged and shipped to Rithron Associates Incorporated for taxonomic identification. Macroinvertebrate samples were identified to the lowest taxonomic level possible, commonly species or genus. Several macroinvertebrates could only be identified to family or subfamily, of which Oligochaeta was the most common.

Taxonomic Diversity

To quantify taxonomic diversity, I calculated two commonly used taxonomic diversity indices, Shannon Diversity Index and Simpson's Diversity Index. The calculation for Shannon Diversity takes into consideration both the abundance and evenness of species present within a location and produces a value representative of species diversity:

$$H' = -\sum_{i=1}^{R} p_i(ln(p_i))$$

Simpson's Diversity was calculated using Simpson's Reciprocal Index so that values could be interpreted similarly to Shannon diversity (i.e. value increases with diversity). Simpson's diversity quantifies the probability that two randomly selected individuals in a sample will belong to the same species and takes into consideration species richness and evenness:

$$D = \frac{1}{\frac{\sum n(n-1)}{N(N-1)}}$$

I also calculated family richness by summing the number of taxonomic families present for a given study site:

$$Family\ Richness = \sum (the\ number\ of\ families\ at\ each\ study\ site)$$

I calculated family richness in lieu of species richness because data included some individuals that could not be identified lower than family level (e.g. Oligochaeta).

In addition, I calculated several other metrics to characterize the macroinvertebrate community (e.g. 1) Percent Ephemeroptera, Plecoptera, and Trichoptera (EPT): the percentage of individuals in the total sample belonging to the described orders, 2) Percent Tribe Tanytarsini Midge Composition: the percentage of individuals in the total sample belonging to the Chironominae midge tribe tanytarsini, 3) Percent Mayfly: the percentage of individuals in the total sample belonging to the order Ephemeroptera, 4) Percent Caddisfly: the percentage of individuals in the total sample belonging to the order Trichoptera, 5) Percent Other Dipterans and Non-Insects: the percentage of individuals in the total sample that do not belong to the tribe tanytarsini along with individuals that are non-insects, and 6) Percent Tolerant: the percentage of individuals in the total sample belonging to a list of known tolerant taxa, contained in the ICI).

These are metrics that are a part of DeShon's Invertebrate Community Index (ICI) (DeShon, 1995). However, I did not calculate overall ICI scores because our methodology did not include sampling required for the final, qualitative metric of this index. I calculated other taxonomic metrics (e.g. 1) Percent Chironomidae: the percentage of individuals in the total sample belonging to the Chironomidae family, 2) EPT/Chironomidae: the ratio of individuals belonging to the Ephemeroptera, Plecoptera, and Trichoptera orders to individuals belonging to the Chironomidae family, and 3) Percent Dominant: the percentage of individuals at each site belonging to the family at each site that contained the greatest number of individuals) based on relationships identified from previous research (Barton & Metcalfe-Smith, 1992; Stewart et al., 2000).

Functional Diversity

To quantify functional diversity, I primarily used functional feeding groups (FFGs). Macroinvertebrates were classified into FFGs based on data obtained from *An Introduction to the Aquatic Insects of North America* (Merritt & Cummins, 2008). I cross-referenced each classification with the United States Environmental Protection Agency's Freshwater Biological Traits Database for accuracy (US EPA National Center for Environmental Assessment, 2017). If macroinvertebrates exhibited more than one feeding behavior, I placed them into a FFG according to their primary behavior. Functional feeding groups utilized were predator, collector-gatherer, collector-filterer, scraper, and shredder. Classifications were used to determine relative abundances of macroinvertebrates in each FFG. I calculated functional feeding group richness by summing the number of FFGs present for a given study site. Functional and taxonomic Shannon and Simpson's diversity were calculated in the same manner with the exception of

substituting the proportion of individuals belonging to a FFG for the proportion of individuals belonging to a taxa.

To explore differences in macroinvertebrate modes of existence (i.e. an organism's method of movement, attachment, or concealment) between watersheds, I classified organisms into categories according to data obtained from *An Introduction to the Aquatic Insects of North America* (Merritt & Cummins, 2008). I calculated the percentage of individuals in the burrower group and the percentage of individuals in the clinger group at each site. Previous research indicated that these two groups could be associated with changes in land use and so they were the only two mode of existence groups for which I calculated proportions (Wagenhoff et al., 2012). All classifications were cross-referenced with the US EPA's Freshwater Biological Traits Database (US EPA National Center for Environmental Assessment, 2017). If an organism exhibited more than one mode of existence, I placed it into the category congruent with the EPA's trait database. Some organisms could not be placed into a single category due to lack of detailed information. If this occurred, I included the organism in the calculation of each mode of existence it exhibited. For example, if I had 3 organisms in a sample that exhibited clinger and burrower qualities, I would include 3 organisms in the burrower calculation and 3 organisms in the clinger calculation. Other research recognizes the potential for an organism to exhibit more than one mode of existence during its lifetime, however, classify the organism into a single category regardless of this. This method of calculating relative abundances can reduce accuracy and reliability when using mode of existence as a functional diversity metric (Heino, 2005).

Statistical Analyses

I used a series of univariate analyses to test for differences in macroinvertebrate diversity, nutrient concentrations, and physical characteristics between watersheds that vary in land use (i.e. Indian Lake, Hoover, Burr Oak). Initial analyses included a series of one-way analysis of variances (ANOVAs) with watershed as the independent variable and each of the different macroinvertebrate diversity metrics, nutrient, or physical characteristic as the dependent variable. I examined the residuals from each ANOVA and used a Shapiro-Wilks test to determine whether residuals were normally distributed. If results of the Shapiro-Wilks test revealed that data were normally distributed and the ANOVA produced a significant p-value, I ran Tukey's HSD test to determine pair-wise differences between watersheds. If results of the Shapiro-Wilks test revealed that residuals were not normally distributed, data were analyzed with the Kruskal-Wallis one-way analysis of variance (non-parametric statistical test). When the Kruskal-Wallis test revealed significant differences, I ran Conover's test of multiple comparisons to reveal pair-wise differences.

Linear regressions were run to evaluate whether taxonomic or functional macroinvertebrate diversity better explained variation in nutrient concentrations across sites. I ran regressions with nutrient (e.g. total nitrogen, total phosphorus) as the independent variable and taxonomic or functional diversity as the dependent variable (i.e. FFG richness, family richness, Shannon diversity, Simpson's diversity). I used the r^2 value to interpret how well nutrient concentrations explained variation in macroinvertebrate diversity indices.

Mathematical corrections were not explored for multiple comparisons because statistical tests had been planned pre-analysis. All statistical analyses were completed using RStudio (RStudio Team, 2015).

Results

Nutrients

Mean total nitrogen (TN) concentrations differed significantly between the three watersheds ($H = 12.088$, $p = 0.0024$, $df = 2$, See Figure 3 and Table 2). Hoover (mean = 0.964, SE = 0.064, n = 11) and Indian Lake (mean = 1.1615, SE = 0.336, n = 11) sites exhibited the highest TN concentrations but were not significantly different from one another (Conover's post hoc, $p > 0.05$). The mean total nitrogen concentration at Burr Oak sites was 0.674 mg/l (SE = 0.036, n = 6), which was significantly lower than TN concentrations at Hoover and Indian Lake (Conover's post hoc, $p < 0.05$).

Mean total phosphorus (TP) concentrations differed significantly between catchments ($H = 10.388$, $p = 0.0056$, $df = 2$, See Figure 4 and Table 2). Similarly to total nitrogen, total phosphorus concentrations were highest at Hoover (mean = 0.067, SE = 0.006, n = 11) and Indian Lake (mean = 0.064, SE = 0.006, n = 11) but the two catchments did not significantly differ from one another (Conover's post hoc, $p > 0.05$). Mean total phosphorus concentration was 0.048 mg/l at Burr Oak sites (SE = 0.001, n = 6), which was significantly lower than TP concentrations at Hoover and Indian Lake sites (Conover's post hoc, $p < 0.05$).

Phosphate (PO_4-P) concentrations exhibited significant differences between watersheds ($H= 8.4142$, $p = 0.0149$, $df = 2$, See Figure 5 and Table 2). Phosphate concentrations were highest at Hoover (mean = 0.033, SE = 0.007, n = 11) and significantly different from Burr Oak (Conover's post hoc, $p < 0.05$) which had the lowest phosphate concentrations at 0.012 mg/l (SE = 0.002, n = 6). Phosphate concentrations at Indian Lake sites (mean = 0.018, SE = 0.003, n = 11) did not significantly differ from Hoover or Burr Oak sites (Conover's post hoc, $p > 0.05$).

The concentrations of nitrate-nitrogen or ammonia-nitrogen did not significantly differ across Hoover, Indian Lake, or Burr Oak watershed (See Tables 2 and 3 for relevant statistics, p = > 0.05).

Physical Characteristics

Mean Qualitative Habitat Evaluation Index (QHEI) scores did not significantly differ between sites, however, QHEI scores trended highest at Hoover (mean = 53.69, SE = 4.33, n = 8) and Burr Oak (mean = 53.58, SE = 4.23, n = 6) and lowest at Indian Lake (mean = 38.77, SE = 4.29, n = 10) ($F_{2,21}$ = 3.1685, p = 0.0627, See Table 4).

Mean Riparian Score differed significantly between catchments ($F_{2,25}$ = 4.5029, p = 0.0214, See Figure 6 and Table 4). Mean Riparian Score was 3.250 at Burr Oak (SE = 0.422, n = 6), which was the highest of all three watersheds. Burr Oak significantly differed from the watershed exhibiting the lowest Riparian Score, Indian Lake (mean = 1.636, SE = 0.364, n = 11) (Conover's post hoc, $p < 0.05$). Hoover sites (mean = 2.727, SE = 0.326, n = 11) did not differ significantly from Indian Lake or Burr Oak (Conover's post hoc, $p > 0.05$).

Taxonomic and Functional Variables

There were no significant differences between mean family richness across watersheds ($F_{2,25}$ = 2.0545, p = 0.1493, See Table 5). Additionally, there were no significant differences in taxonomic Shannon diversity ($F_{2,25}$ = 0.0303, p = 0.9702, See Table 5) or taxonomic Simpson's diversity (H = 0.3283, p = 0.8486, See Table 6) across catchments. Furthermore, the additional taxonomic variables explored (e.g. Percent EPT, Taxa Richness, Percent Mayfly, Percent Caddisfly, Percent Tribe Tanytarsini, Percent Other Dipteran/Non-insect, Percent Tolerant,

Percent Chironomidae, EPT/Chironomidae, Percent Dominant Species) did not reveal significant differences across watersheds (See Table 5 and Table 6 for relevant statistics, $p > 0.05$).

Mean functional feeding group richness (FFG) differed significantly between catchments ($F_{2,25} = 5.2499$, $p = 0.0125$, See Figure 7 and Table 7) and was highest at Indian Lake (mean = 4.09, SE = 0.251, n=11). Burr Oak sites exhibited significantly lower mean FFG richness (mean = 2.67, SE = 0.42, n = 6) than Indian Lake sites (Tukey's HSD, $p < 0.05$). Hoover sites (mean = 3.75, SE = 0.244, n = 11) were not significantly different from Indian Lake or Burr Oak (Tukey's HSD, $p > 0.05$).

The mean percentage of individuals in the collector-filterer (CF) feeding group was significantly different between watersheds ($H = 6.5056$, $p = 0.0387$, $df = 2$, See Figure 8 and Table 8) and highest at Hoover (mean = 10.37, SE = 4.13, n=11) and Indian Lake (mean = 8.98, SE = 6.18, n=11). The two catchments, however, did not have significant separation of means (Conover's post hoc, $p > 0.05$). Burr Oak (mean = 0.260, SE = 0.260, n = 6) had a significantly lower percentage of CFs than Indian Lake or Hoover sites (Conover's post hoc, $p < 0.05$).

The mean percentage of individuals in the predator feeding group differed significantly between catchments ($H = 8.5924$, $p = 0.0136$, $df = 2$, See Figure 9 and Table 8). Burr Oak exhibited the highest mean percentage of predator individuals (mean = 42.50, SE = 11.68, n = 6). Indian Lake (mean = 6.45, SE = 1.26, n = 11) and Hoover (mean = 6.77, SE = 1.61, n = 11) sites contained significantly fewer predators than Burr Oak (Conover's post hoc, $p < 0.05$), however, the percentage of predator individuals did not differ between Hoover and Indian Lake (Conover's post hoc, $p > 0.05$).

The remaining functional metrics explored (e.g. Percent Scraper, Percent Shredder, Percent Collector-gatherer, Scraper/Collector-filterer, Shredder/Total Individuals, Percent Burrower, Percent Clinger) did not significantly differ across watersheds (Refer to Table 7 and Table 8 for relevant statistics, $p = >0.05$).

Linear Regressions

Family richness was significantly and positively correlated with total phosphorus ($F_{1,26} = 5.6780$, $p = 0.0248$, $r^2 = 0.1792$, See Figure 10 and Table 9) and phosphate ($F_{1,26} = 7.6570$, $p = 0.0103$, $r^2 = 0.2275$, See Figure 11 and Table 9) but was not significantly correlated with total nitrogen ($F_{1,26} = 0.2868$, $p = 0.7544$, $r^2 = 0.0038$), nitrate-nitrogen ($F_{1,26} = 0.3390$, $p = 0.5654$, $r^2 = 0.0129$), or ammonia-nitrogen ($F_{1,26} = 2.4700$, $p = 0.1281$, $r^2 = 0.0868$). Taxonomic Shannon diversity was not significantly correlated with total nitrogen ($F_{1,26} = 0.1000$, $p = 0.7544$, $r^2 = 0.0038$), total phosphorus ($F_{1,26} = 0.6340$, $p = 0.4331$, $r^2 = 0.0238$), phosphate ($F_{1,26} = 0.6340$, $p = 0.4331$, $r^2 = 0.0238$), nitrate-nitrogen ($F_{1,26} = 0.0061$, $p = 0.9383$, $r^2 = 0.0002$), or ammonia-nitrogen ($F_{1,26} = 2.4700$, $p = 0.1281$, $r^2 = 0.0868$) (See Table 9). Taxonomic Simpson's diversity was not significantly correlated with total nitrogen ($F_{1,26} = 0.6671$, $p = 0.4215$, $r^2 = 0.0250$), total phosphorus ($F_{1,26} = 2.3570$, $p = 0.1368$, $r^2 = 0.0831$), phosphate ($F_{1,26} = 1.0400$, $p = 0.3172$, $r^2 = 0.0385$), nitrate-nitrogen ($F_{1,26} = 0.3874$, $p = 0.5391$, $r^2 = 0.0147$), or ammonia-nitrogen ($F_{1,26} = 0.2076$, $p = 0.6524$, $r^2 = 0.0079$) (See Table 9).

Functional feeding group richness was significantly and positively correlated with total phosphorus concentration ($F_{1,26} = 5.5970$, $p = 0.0257$, $r^2 = 0.1771$) but was not significantly correlated with total nitrogen ($F_{1,26} = 1.9680$, $p = 0.1725$, $r^2 = 0.0704$), phosphate ($F_{1,26} = 1.0290$, $p = 0.3197$, $r^2 = 0.0381$), nitrate-nitrogen ($F_{1,26} = 2.0240$, $p = 0.1667$, $r^2 = 0.0722$), or ammonia-

nitrogen ($F_{1,26} = 0.8171$, $p = 0.3743$, $r^2 = 0.0305$) (See Figure 12 and Table 10). Functional Shannon diversity was not significantly correlated with total nitrogen ($F_{1,26} = 0.0017$, $p = 0.9676$, $r^2 = 0.0001$), total phosphorus ($F_{1,26} = 0.0383$, $p = 0.8465$, $r^2 = 0.0015$), phosphate ($F_{1,26} = 0.0653$, $p = 0.8003$, $r^2 = 0.0025$), nitrate-nitrogen ($F_{1,26} = 0.1612$, $p = 0.6914$, $r^2 = 0.0062$), or ammonia-nitrogen ($F_{1,26} = 0.4353$, $p = 0.5152$, $r^2 = 0.0165$) (See Table 10). Functional Simpson's diversity was not significantly correlated with total nitrogen ($F_{1,26} = 0.2047$, $p = 0.6547$, $r^2 = 0.0078$), total phosphorus ($F_{1,26} = 0.1411$, $p = 0.7103$, $r^2 = 0.0054$), phosphate ($F_{1,26} = 0.0459$, $p = 0.8321$, $r^2 = 0.0018$), nitrate-nitrogen ($F_{1,26} = 0.0165$, $p = 0.8989$, $r^2 = 0.0063$), or ammonia-nitrogen ($F_{1,26} = 0.1528$, $p = 0.6990$, $r^2 = 0.0058$) (See Table 10).

Discussion

Overall, results of nutrient analyses validated differences across watersheds with varying land use as predicted. As expected, the mixed-use and agricultural sites contained the highest concentrations of total nitrogen, total phosphorus, and phosphate (Figures 3,4,5) and the forested catchment consistently exhibited the lowest nutrient concentrations (i.e. total nitrogen, total phosphorus, and phosphate). There were no significant differences in nitrate-nitrogen and ammonia-nitrogen across watersheds, but there was a tendency for nitrate-nitrogen concentrations to be higher at Indian Lake and Hoover in comparison to Burr Oak ($p = 0.0660$). Contrary to what I predicted, there were no significant differences in family richness, Shannon's diversity (e.g. taxonomic or functional) or Simpson's diversity (e.g. taxonomic or functional) across watersheds. However, there was a significant difference in functional feeding group richness across catchments. The agricultural and mixed-use watersheds had higher functional feeding group richness than the forested (Figure 7), antithetical to my prediction. The percentage

of collector-filterers differed significantly across watersheds with the highest percentages present at Indian Lake and Hoover and the lowest at Burr Oak (Figure 8). Additionally, the percentage of predator taxa significantly differed between catchments. Burr Oak exhibited a higher percentage of predators in comparison with Hoover and Indian Lake (Figure 9). Interestingly, looking at the general relationship between nutrients and macroinvertebrate biodiversity across watersheds, linear regressions revealed that taxonomic family richness was significantly and positively correlated with phosphate. Both family richness and functional feeding group richness were significantly and positively correlated with total phosphorus (Figures 10,11,12). While the results do not provide support of the prediction that macroinvertebrate diversity declines with increasing nutrient concentrations, there is evidence that nutrients could be driving significant shifts in community composition.

Despite the absence of differences in taxonomic diversity and richness as well as some functional diversity metrics across watersheds, linear regressions revealed a positive relationship between nutrients and richness. Results indicated that taxonomic richness was positively correlated with phosphate and phosphorus and functional richness was positively correlated with phosphorus. Because phosphorus is known as a limiting nutrient in aquatic freshwater systems, the levels of phosphate and phosphorus at the study sites could be acting as a subsidy to the macroinvertebrate community (OEPA, 1999). Feuchtmayer et al. (2007) found that consistent exposure to moderate levels of phosphorus increased productivity in some macroinvertebrate taxa (Feuchtmayr, McKee, Harvey, Atkinson, & Moss, 2007). Other research has detected non-linear relationships between macroinvertebrate diversity and nutrient concentrations. Wagenhoff et al. (2012) found evidence of a subsidy-stress relationship between Ephemeroptera, Plecoptera, and Trichoptera (EPT) diversity and nutrients (e.g. nitrogen and phosphorus), such that density

of EPT taxa increased with moderate in-stream nutrient additions but declined when nutrient additions became high (Wagenhoff et al., 2012). A similar, non-linear relationship may have been revealed in the three study watersheds with the collection of additional data points. Another possible explanation could be that the phosphorus concentrations at each site were not high enough to evoke a stress response from the macroinvertebrate community. Supporting this claim, the Ohio Environmental Protection Agency's (OEPA) biologically safe recommendation (e.g. to prevent stress responses in aquatic organisms) for phosphorus in wadeable streams (20 mi^2 < drainage area < 200 mi^2) is 0.11 mg/l (OEPA, 1999). Highest at Hoover, mean total phosphorus concentrations were almost half of the OEPA's recommendation (0.067±0.006 mg/l) and phosphate concentrations were even lower (0.033±0.024 mg/l). Despite the relationship between richness and nutrients (e.g. phosphorus and phosphate) being contrary to my predictions, closer examination of functional groups provides a better indication of the dynamics of the invert communities at each site.

There was a significant difference in the percentage of macroinvertebrates in the collector-filterer feeding group across watersheds. The percentage of collector-filterers was highest in the agricultural and mixed use catchments and lowest in the forested catchment (Figure 8). Collector-filterers feed on fine particulate organic matter (e.g. organic material <1mm in diameter) and can easily exploit resources (e.g. nutrients, algal slough, etc.) found in the water column while conserving energy for biomass (Wallace & Webster, 1996). Research by Stewart et al. (2000) found that the highest percentage of the functional feeding group, collector-gatherers, existed at sites that had been most heavily impacted by changes in land use (e.g. increased nutrients, reduction of allochthonous inputs) (Stewart et al., 2000). Similar to collector-filterers, collector-gatherers rely on the same autochthonous sources of energy but use a

different method of resource acquisition (e.g. mouthpart morphology) (Merritt & Cummins, 2008). This provides evidence that collector-filterers may benefit from increased autochthonous production and nutrient inputs resulting from development of land. Other research by Wallace and Webster (1996) suggest that agricultural conversion of land facilitates bottom-up effects, which causes significant increases in autochthonous inputs. This shift in available resources increases the proportion of collector-filterers in a given stream reach (Wallace & Webster, 1996). Interestingly, mean riparian score was lowest in the agricultural watershed, suggesting that Indian Lake streams derive a large portion of their energy from autochthonous sources. This data corroborates visual observations made at the study sites. The high percentage of collector-filterers observed at Indian Lake and Hoover could be attributed to a shift towards autochthonous energy inputs associated with changes in land use. This is analogous to what has been observed in the primary literature.

One of the most intriguing results revealed by the functional diversity analyses was a significant difference in the mean percentage of predator taxa across watersheds. The percentage of predator taxa was highest at Burr Oak and lowest at Hoover and Indian Lake (Figure 9). Recall that mean functional feeding group richness was the opposite (e.g. highest at Indian Lake and lowest at Burr Oak) (Figure 7). Because functional feeding group richness was highest at Indian Lake and Hoover, I would expect these two watersheds to support a more trophically-diverse community than Burr Oak (Hooper D. U. et al., 2005). Reductions in riparian width could be responsible for a greater proportion of generalist species (e.g. collector-filterers, collector-gatherers) at Indian Lake and Hoover. This could be driving the high functional richness values observed at these catchments. The large proportion of generalist species (e.g. collector-filterers, collector-gatherers) could be placing stress on the carrying capacity of the

macroinvertebrate community which in turn negatively impacts Hoover and Indian Lake's ability to support higher trophic levels (e.g. predators). Alternatively, research by Wallace and Webster (1996) suggests that diverse fish assemblages can cause significant decreases in macroinvertebrate predator abundances (Wallace & Webster, 1996). Other research by Feuchtmayer et al. (2007) found that diversity of macroinvertebrate communities decreased in the presence of fish (Feuchtmayr et al., 2007). When sampling occurred at Burr Oak in 2016, surface water was below base level and sampling could not be completed as planned at five sites. Although fish data were not included in this study, fluctuations in water levels at Burr Oak could be driving top-down effects and releasing pressure on macroinvertebrate predators.

Opposite my predictions, there were no other significant relationships between diversity metrics (e.g. functional and taxonomic) across watersheds. One reason that relationships between these two variables were not more evident could be that data only contained one collection effort (e.g. nutrients, macroinvertebrates, physical habitat) at each site. Adding data from additional sampling efforts (e.g. seasons, years, etc.) could help to reduce variation in metrics and reveal significant differences in diversity across watersheds (e.g. agricultural, mixed-use, and forested). While each watershed contained multiple collection sites, the unit of replication for each land use type (e.g. agricultural, mixed-use, and forested) was one. Collecting additional data from other watersheds exhibiting the same primary land use provide support for my conclusions. Riparian score could be responsible for driving some of the variation in macroinvertebrate diversity across land use types. Future analyses should include the addition of riparian score and substrate size to the model to account for their potential effect on community composition across land use types. Additionally, research has suggested that Shannon and Simpson's diversity may be extremely responsive to the type of macroinvertebrate

collection method utilized (Charvet et al., 1998). According to Merritt and Cummins (2008), the two methods used in this study for macroinvertebrate sampling (e.g. Surber and Ekman) are the two most commonly used pieces of equipment. I do not believe that the type of sampling equipment used in this study had an impact on diversity values because sampling methods were consistent across the three watersheds. In the event that variation in communities did occur, this variation would be present in all samples.

In conclusion, the use of functional diversity metrics allowed for a greater level of detail when describing the macroinvertebrate community and aided in the identification of potential environmental stressors responsible for the observed patterns of diversity. Traditional taxonomic-based measures of diversity were not as responsive to shifts in nutrient concentrations and land use changes. The use of functional feeding groups (e.g. predators, shredders, collector-gatherers, collector-filterers, scrapers) provided a possible explanation for the community dynamics observed within each watershed. Additional, multivariate analyses could provide an even greater level of detail. Furthermore, functional feeding group data was easy to gather, percentages were easy to calculate, and metrics were readily comparable with other data sets (e.g. nutrient concentrations and physical characteristics). Integrating functional diversity metrics (e.g. feeding groups) with traditional taxonomic indices could make areas of impairment within watersheds easier to identify which could allow for smaller-scale ecosystem restoration projects with higher rates of success and less economic impact. This research provides support for the integration of functional diversity metrics in macroinvertebrate biomonitoring efforts to increase understanding of the effects of land use on aquatic communities.

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Appendix A: Tables

Table 1

Riparian scoring system used to quantify riparian width based on data obtained in the Qualitative Habitat Evaluation Index (QHEI).

Riparian Width (from QHEI)	Score
None	0
Very Narrow (<5m)	1
Narrow (5-10m)	2
Moderate (10-50m)	3
Wide (>50m)	4

Table 2

Results of Kruskal-Wallis tests to determine significant differences between inorganic nutrient concentrations and watershed. (*) P < 0.05

	H	p	df
Total Phosphorus	***10.388***	***0.0056****	***2***
Total Nitrogen	***12.088***	***0.0023****	***2***
Phosphate-Phosphorus	***8.4142***	***0.01489****	***2***
Nitrate-Nitrogen	5.4355	0.0660	2

Table 3

Results of one-way analysis of variances to determine significant differences between inorganic nutrient concentrations and watershed.

	F	p	df
Ammonia-Nitrogen	0.2648	0.7695	2,25

Table 4

Results of one-way analysis of variances to determine significant differences between physical characteristics and watershed. (*) P <0.05

	F	p	df
QHEI	3.1685	0.0627	2,21
Riparian Score	***4.5029***	***0.0214***	***2,25***

Table 5

Results of a one-way analysis of variance to determine significant differences between metrics of taxonomic diversity and watershed.

	F	p	df
Taxonomic Shannon Diversity	0.0303	0.9702	2,25
Family Richness	2.0545	0.1493	2,25
Percentage of Other Dipteran or Non-Insect Individuals	1.0735	0.3570	2,25
Percentage of Individuals Belonging to Dominant Family	2.7215	0.0852	2,25

Table 6

Results of Kruskal-Wallis tests to determine significant differences between taxonomic metrics of diversity and watershed.

	H	p	df
Taxonomic Simpsons Diversity	0.3283	0.8486	2
Percentage of EPT Individuals	2.7432	0.2537	2
Taxa Richness	2.1268	0.3453	2
Percentage of Mayfly Individuals	1.8183	0.4029	2
Percentage of Caddisfly Individuals	2.0348	0.3615	2
Percentage of Tribe Tanytarsini Individuals	2.4891	0.2881	2
Percentage of Tolerant Individuals	1.1498	0.5628	2
Percentage of Chironomidae Individuals	1.7211	0.4229	2
Ratio of EPT/Chironomidae Individuals	2.8250	0.2435	2

Table 7

Results of one-way analysis of variances to determine significant differences between functional diversity metrics and watershed. (*) P < 0.05

	F	p	df
Functional Feeding Group Richness	***5.2499***	***0.0125****	***2,25***
Functional Shannon Diversity	1.0047	0.3805	2,25
Functional Simpsons Diversity	2.3668	0.1145	2,25
Percentage of Collector-Gatherer Individuals	2.9837	0.6886	2,25
Percentage of Burrower Individuals	0.3332	0.7198	2,25
Percentage of Clinger Individuals	0.4028	0.6727	2,25

Table 8

Results of Kruskal-Wallis tests to determine significant differences between functional diversity metrics and watershed (*) P < 0.05

	H	p	df
Percentage of Scraper Individuals	5.5682	0.0618	2
Percentage of Shredder Individuals	1.6925	0.4290	2
Percentage of Collector-Filterer Individuals	***6.5056***	***0.0387****	***2***
Percentage of Predator Individuals	***8.5924***	***0.0136****	***2***
Ratio of Scraper/Collector-Filterer Individuals	3.2405	0.1978	2
Ratio of Shredder/Total Individuals	1.1362	0.5666	2

Table 9

Results of linear regressions between inorganic nutrient concentrations and measures of taxonomic diversity. (*) P < 0.05

	F	p	r^2	df
Taxonomic Shannon Diversity * TN	0.1000	0.7544	0.0038	1,26
Taxonomic Simpson Diversity * TN	0.6671	0.4215	0.0250	1,26
Family Richness * TN	0.2868	0.5968	0.0109	1,26
Taxonomic Shannon Diversity * TP	0.6340	0.4331	0.0238	1,26
Taxonomic Simpson Diversity * TP	2.3570	0.1368	0.0831	1,26
Family Richness * TP	***5.6780***	***0.0248****	***0.1792***	***1,26***
Taxonomic Shannon Diversity * Phosphate	0.6340	0.4331	0.0238	1,26
Taxonomic Simpson Diversity * Phosphate	1.0400	0.3172	0.0385	1,26
Family Richness * Phosphate	***7.6570***	***0.0103****	***0.2275***	***1,26***
Taxonomic Shannon Diversity * Nitrate-N	0.0061	0.9383	0.0002	1,26
Taxonomic Simpson Diversity * Nitrate-N	0.3874	0.5391	0.0147	1,26
Family Richness * Nitrate-N	0.3390	0.5654	0.0129	1,26
Taxonomic Shannon Diversity * Ammonia-N	2.4700	0.1281	0.0868	1,26
Taxonomic Simpson Diversity * Ammonia-N	0.2076	0.6524	0.0079	1,26
Family Richness * Ammonia-N	2.4700	0.1281	0.0868	1,26

Table 10

Results of linear regressions between inorganic nutrient concentrations and measures of functional diversity. (*) P < 0.05

	F	p	r^2	df
Functional Shannon Diversity * TN	0.0017	0.9676	0.0001	1,26
Functional Simpson Diversity * TN	0.2047	0.6547	0.0078	1,26
Functional Feeding Group Richness * TN	1.9680	0.1725	0.0704	1,26
Functional Shannon Diversity * TP	0.0383	0.8465	0.0015	1,26
Functional Simpson Diversity * TP	0.1411	0.7103	0.0054	1,26
Functional Feeding Group Richness * TP	***5.5970***	***0.0257****	***0.1771***	***1,26***
Functional Shannon Diversity * Phosphate	0.0653	0.8003	0.0025	1,26
Functional Simpson Diversity * Phosphate	0.0459	0.8321	0.0018	1,26
Functional Feeding Group Richness * Phosphate	1.0290	0.3197	0.0381	1,26
Functional Shannon Diversity * Nitrate-N	0.1612	0.6914	0.0062	1,26
Functional Simpson Diversity * Nitrate-N	0.0165	0.8989	0.0063	1,26
Functional Feeding Group Richness * Nitrate-N	2.0240	0.1667	0.0722	1,26
Functional Shannon Diversity * Ammonia-N	0.4353	0.5152	0.0165	1,26
Functional Simpson Diversity * Ammonia-N	0.1528	0.6990	0.0058	1,26
Functional Feeding Group Richness * Ammonia-N	0.8171	0.3743	0.0305	1,26

Appendix B: Figures

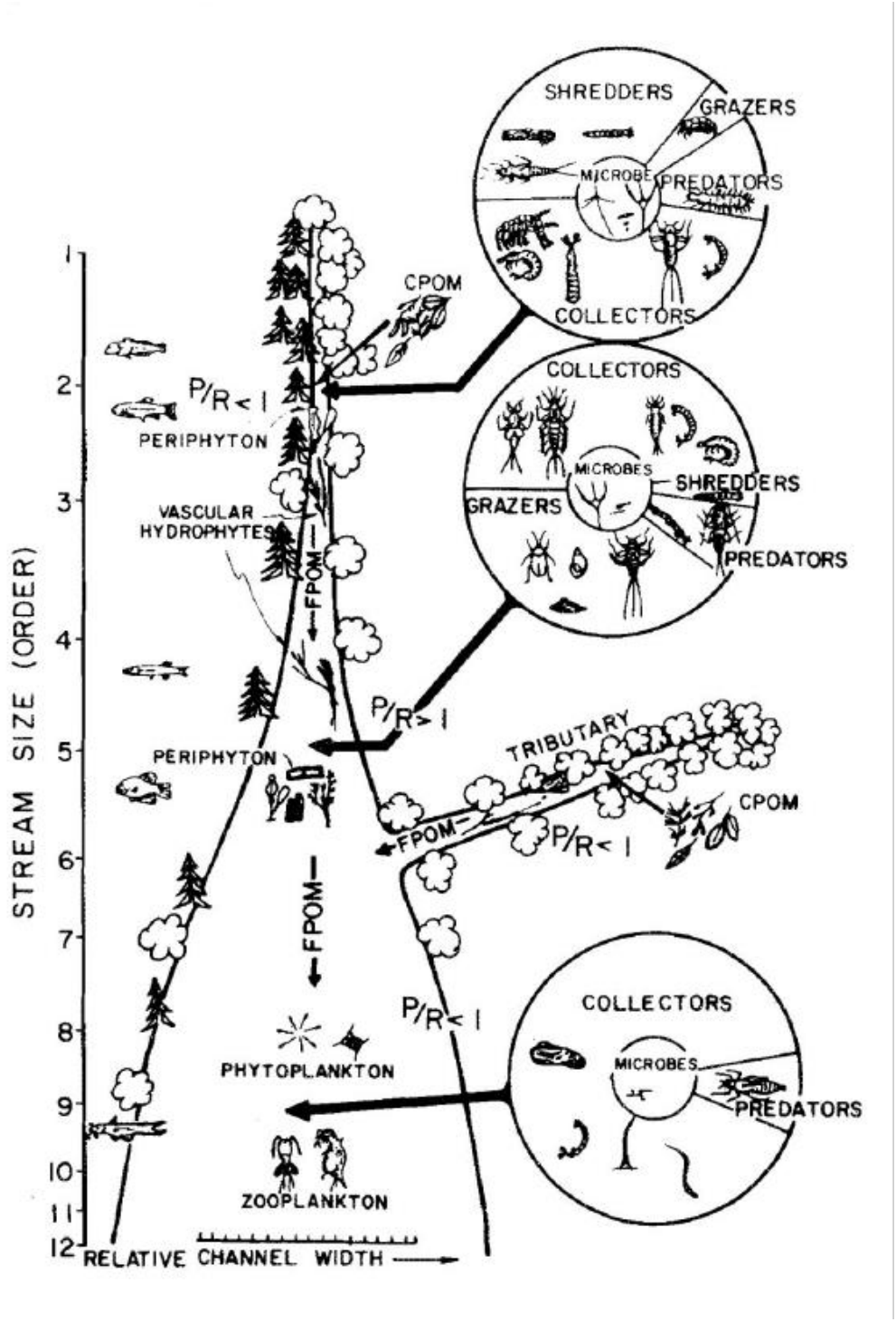

Figure 1. The River Continuum Concept as illustrated by and featured in Vannote et al. (1980).

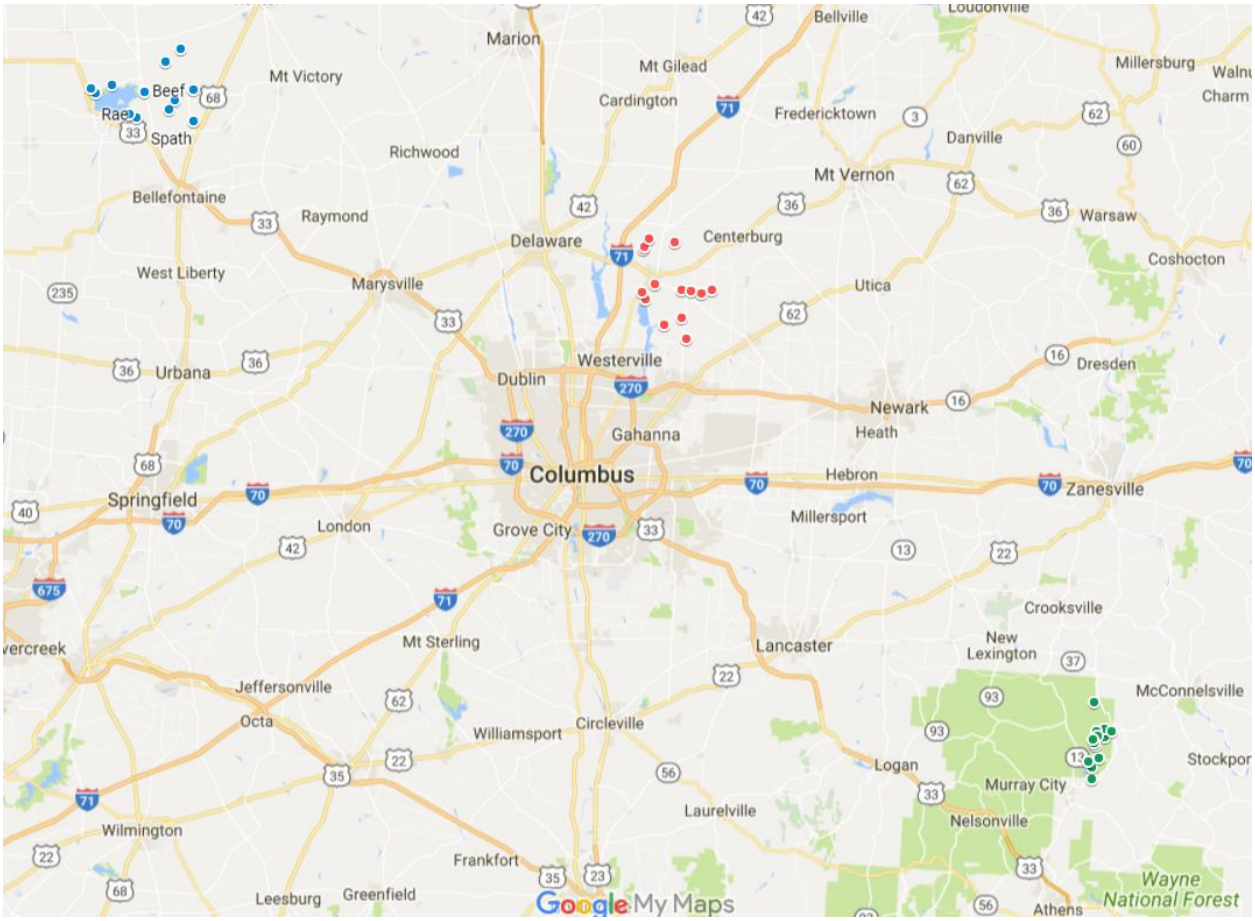

Figure 2. **Study watersheds. Indian Lake sites are represented by the blue dots, Hoover sites are represented by the red dots, and Burr Oak sites are represented by the green dots. Image captured from Google Maps ("Google Maps," n.d.).**

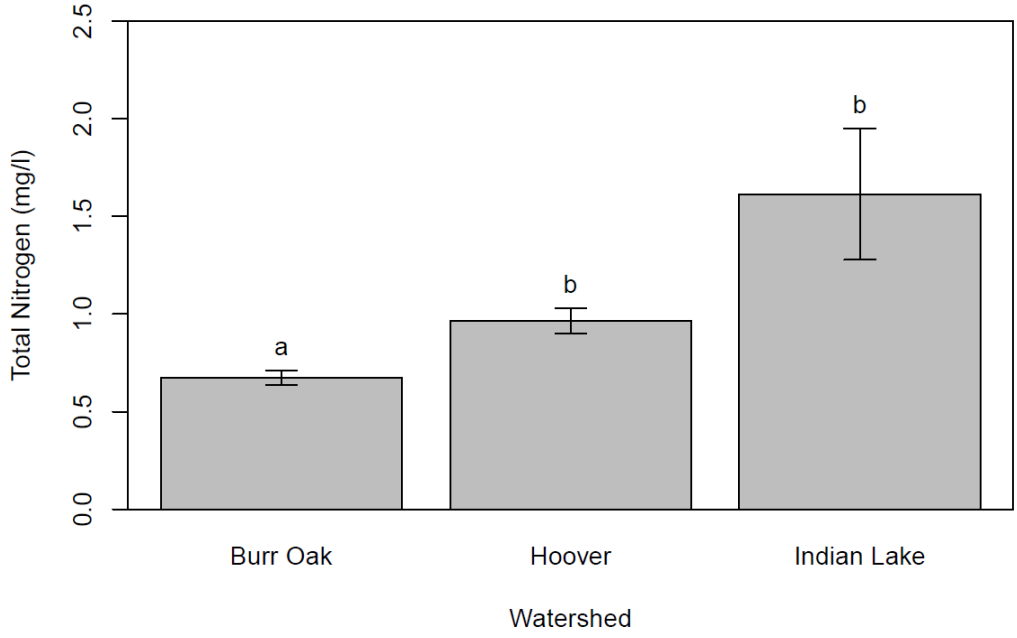

Figure 3. **Mean Total Nitrogen Concentration (mg/l) by Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

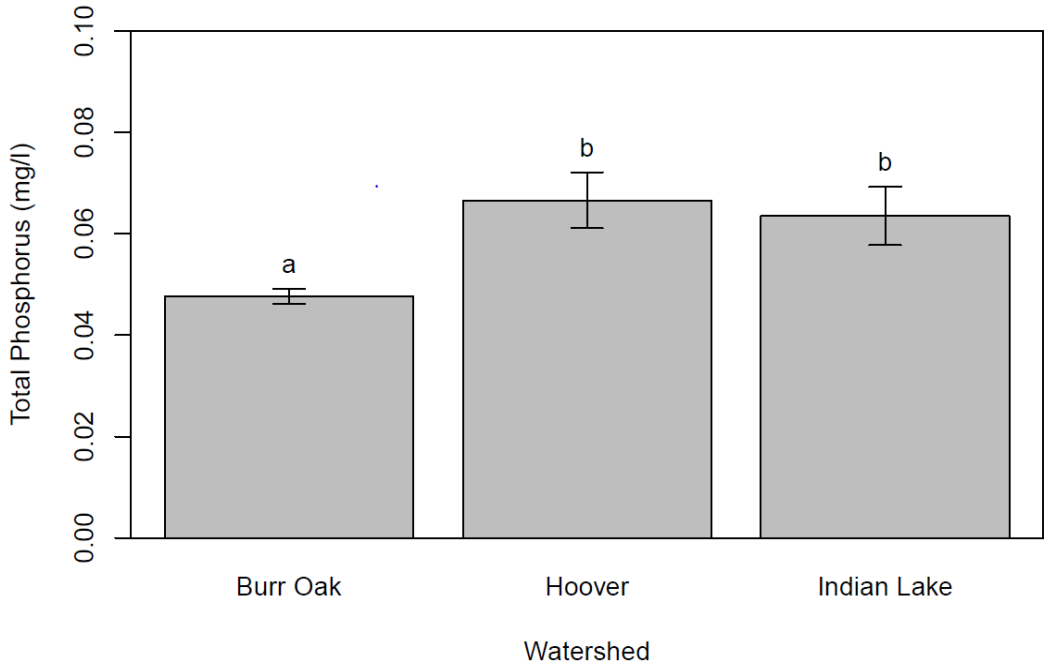

Figure 4. **Mean Total Phosphorus Concentration (mg/l) by Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

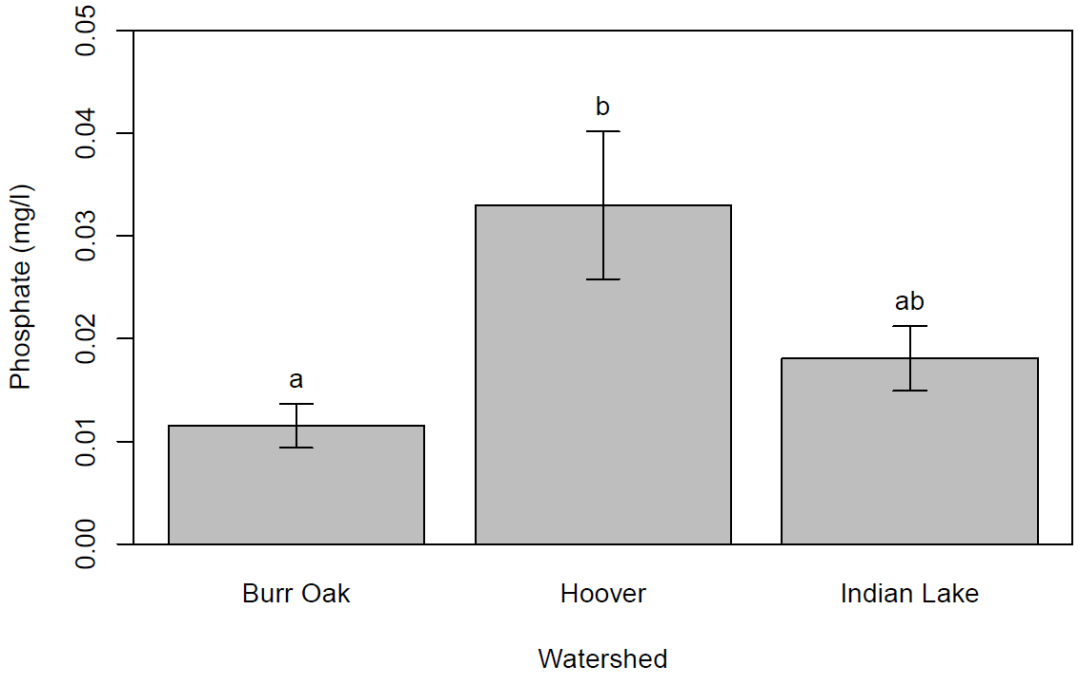

***Figure 5*. Mean Phosphate Concentration (mg/l) by Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

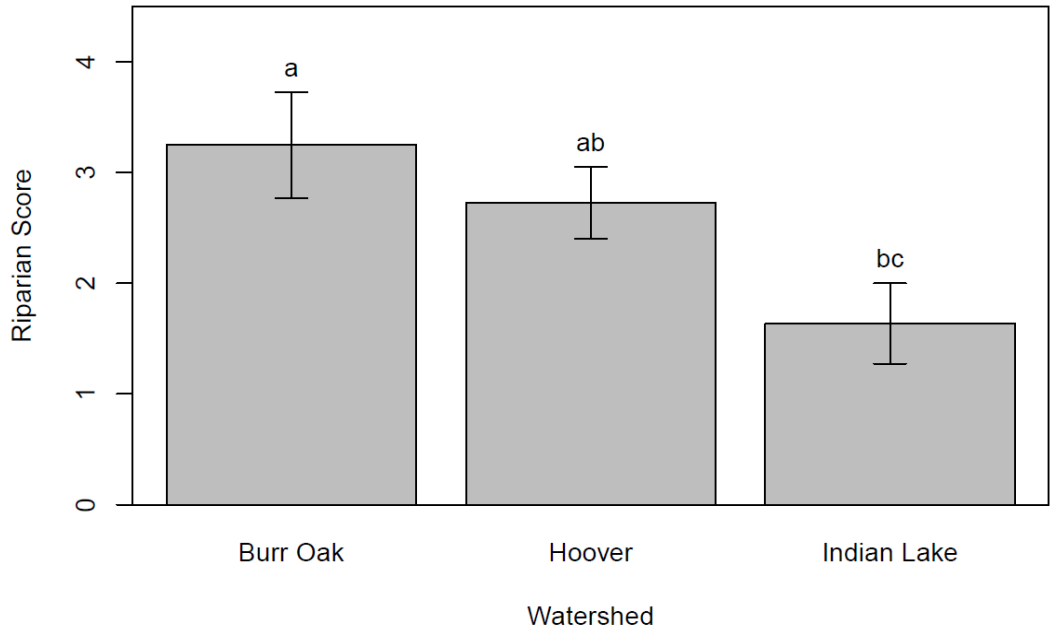

***Figure 6.* Mean Riparian Score by Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

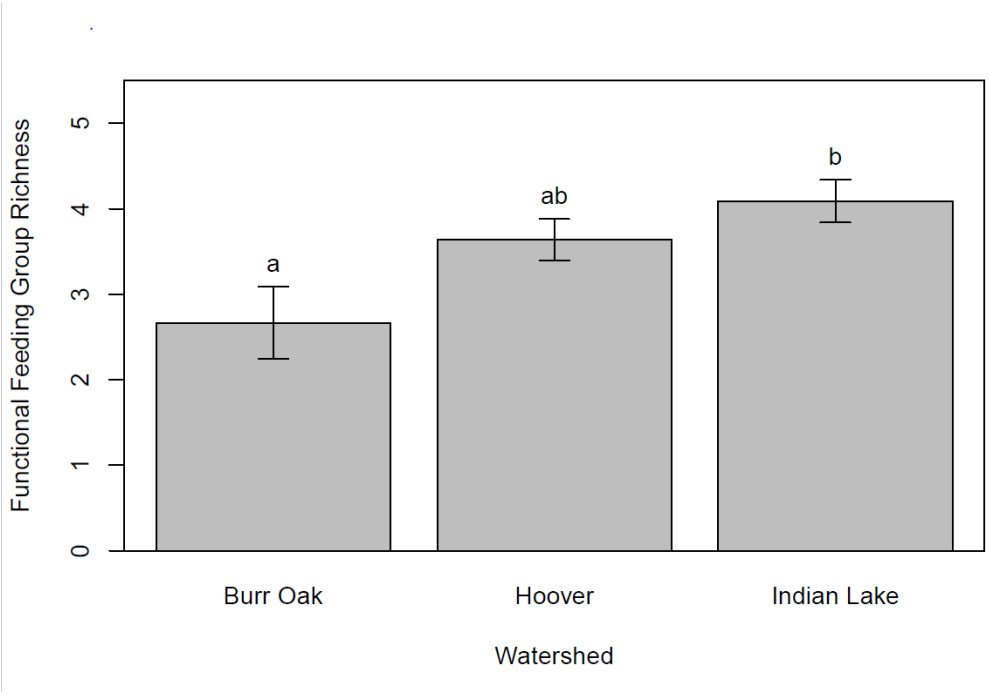

***Figure 7*. Mean Functional Feeding Group Richness (FFG) by Watershed. Letters above bars indicate significant differences in means (Tukey's HSD, $p < 0.05$). Error bars represent standard error.**

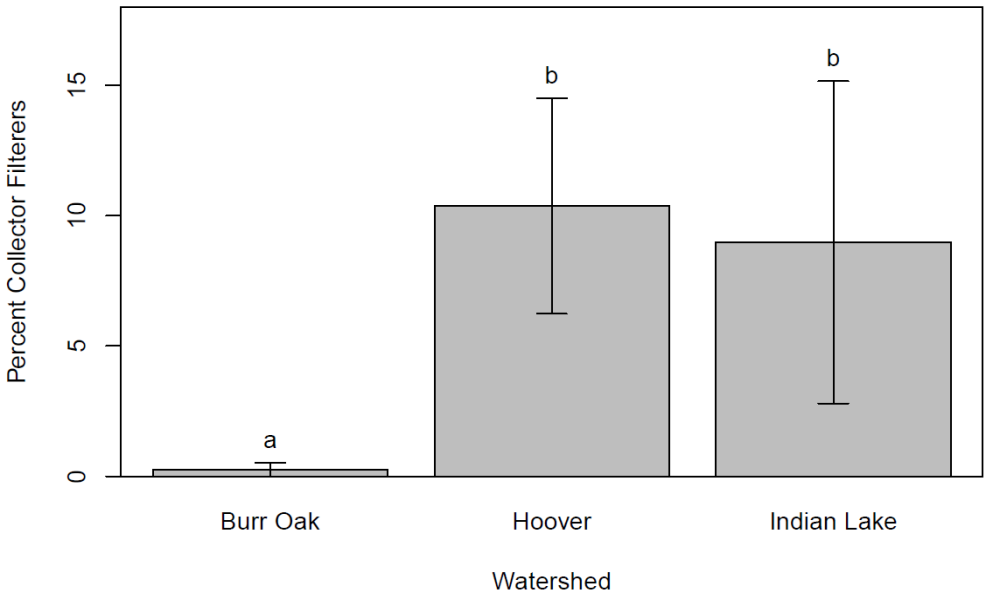

***Figure 8*. Mean Percentage of Collector-Filterers (CFs) per Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

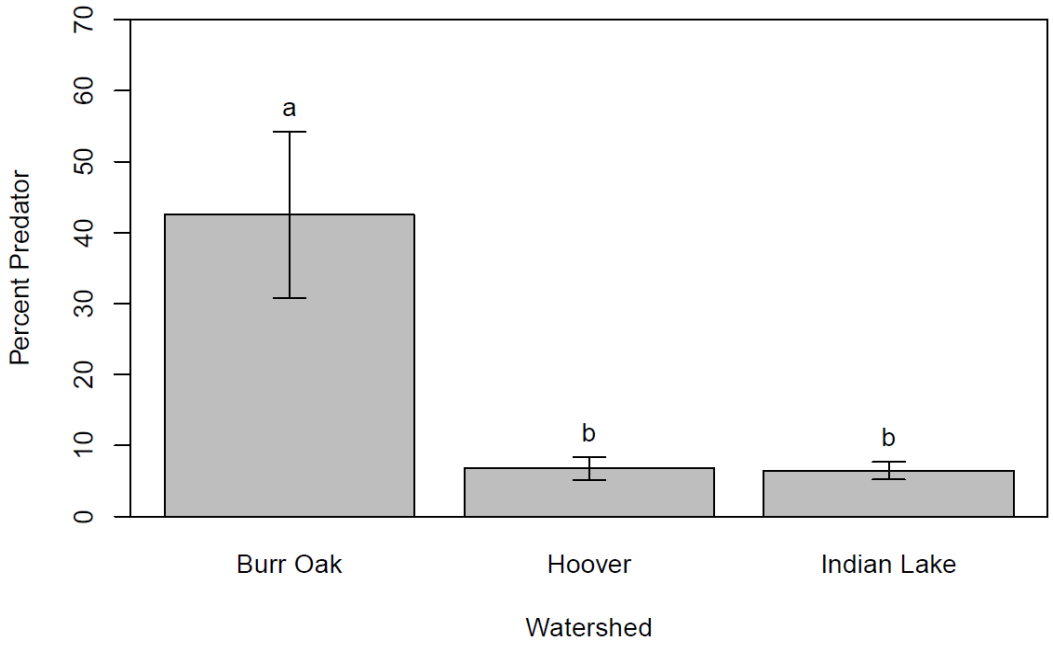

***Figure 9*. Mean Percentage of Predators per Watershed. Letters above bars indicate statistically significant differences in means (Conover's post hoc, $p < 0.05$). Error bars represent standard error.**

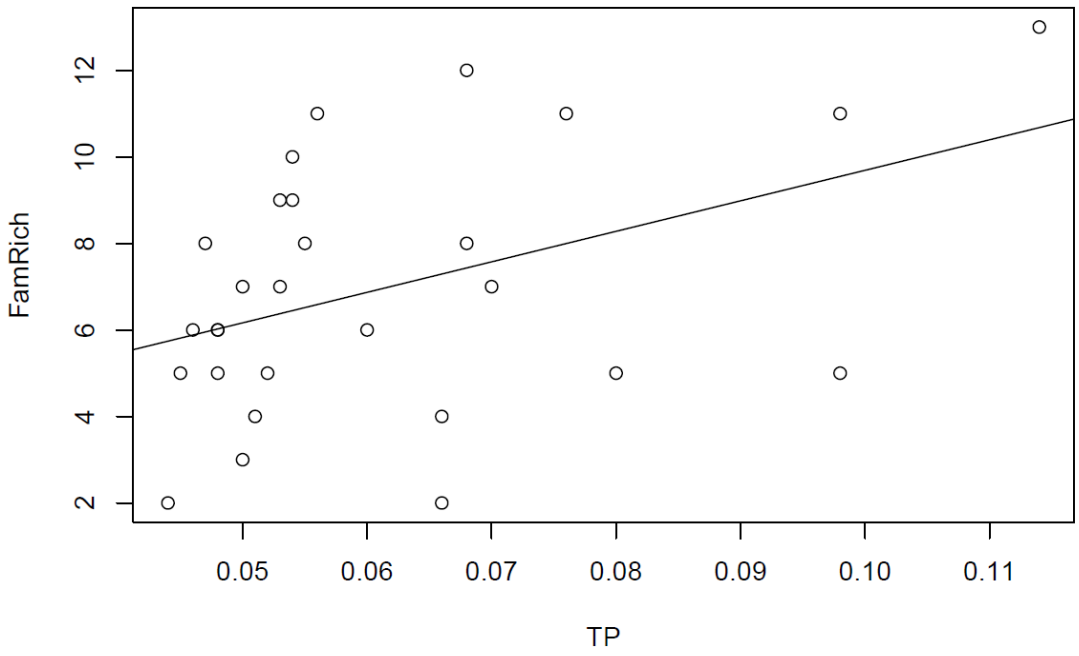

***Figure 10*. Linear Relationship Between Taxonomic Family Richness and Total Phosphorus Concentration (mg/l) (r2 = 0.1792).**

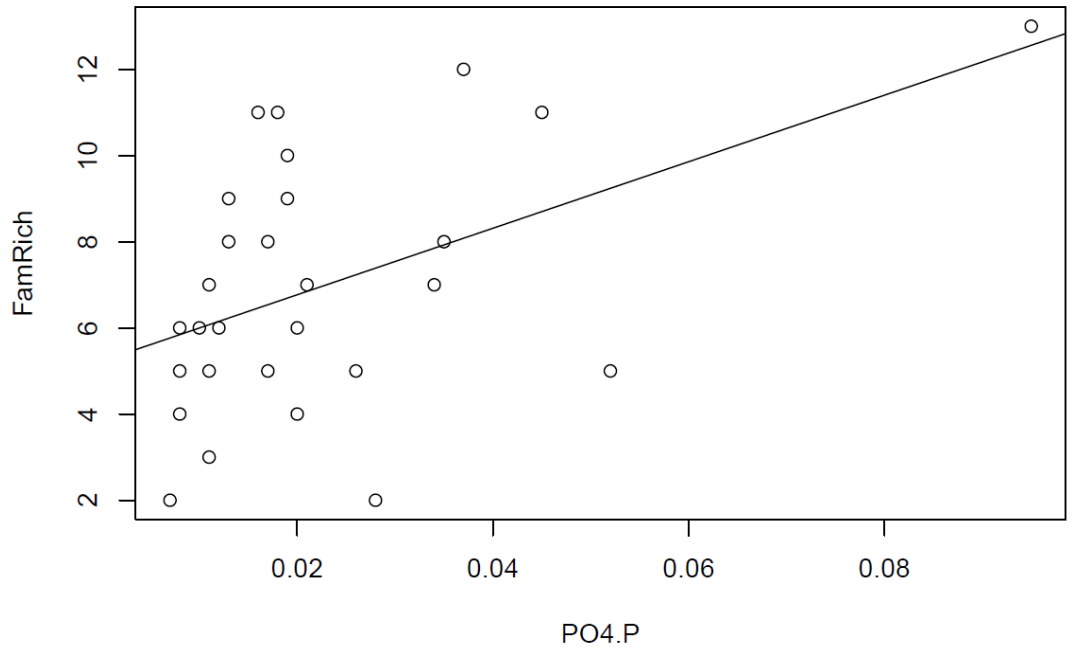

***Figure 11*. Linear Relationship Between Taxonomic Family Richness and Phosphate Concentration (mg/l) (r2 = 0.2275).**

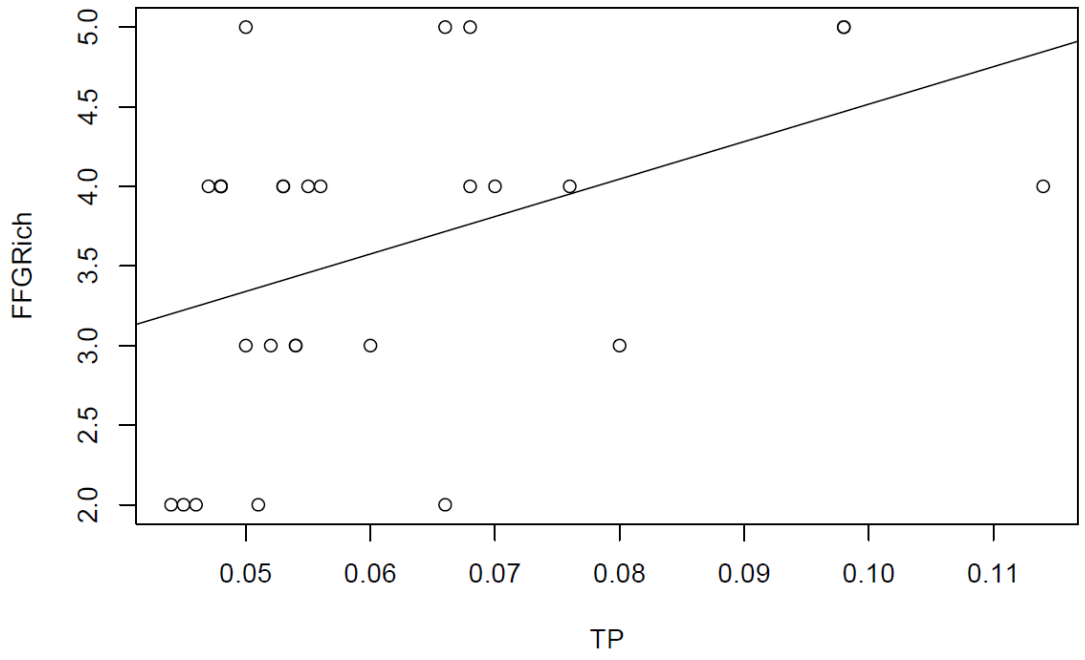

***Figure 12*. Linear Relationship Between Functional Feeding Group Richness and Total Phosphorus Concentrations (mg/l) (r2 = 0.1771).**